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Extractive Determination of Thebaine and Papaverine in Opium

In continuation of the international programme of scientific research on opium undertaken pursuant to resolutions 159 II C (VII) and 246 F (IX), adopted by the Economic and Social Council on 3 August 1948 and 6 July 1949 respectively, the Secretary-General is presenting the following paper prepared by the chemists of the United Nations Secretariat. These new methods for thebaine and papaverine are expected to be useful in the commercial evaluation of different types of opium, in control over the production of opium alkaloids, and in the problem of determining the origin of illicit opium by scientific means.

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I N D E X

	Paragraphs
Introduction	1 - 6
Discussion	7 - 19
Principles of the Thebaine separation	20 - 28
Principles of the Papaverine separation	29 - 33
Charts Summarizing the Method	34
Reagents used for Colour Reactions in the Procedure	35 - 43
Caution concerning Carbon Tetrachloride	44
Detailed Procedure for Thebaine and Papaverine in Opium	45 - 83
Isolation of Thebaine	47 - 62
Determination of the non-thebaine alkaloids in the titrated thebaine residue	63 - 68
Determination of Papaverine in the Chloroform from the Thebaine Determination	69 - 77
Procedure for Papaverine alone	78 - 83
Results	84
Concluding Discussion	85 - 88

Charts 1, 2, and 3 following paragraph 34

Table I following paragraph 84

EXTRACTIVE DETERMINATION OF THEBAINE AND PAPAVERINE IN OPIUM

Introduction

1. The five principal alkaloids of opium, in amount, are morphine, narcotine, codeine, thebaine, and papaverine. The four economic alkaloids of opium are the same with the exception of narcotine, which has very little known value as yet.
2. Methods have been previously given in this series for the determination of morphine, narcotine, and codeine in opium. The determination of thebaine and papaverine therefore completes the determination of the five alkaloids present in the largest amounts, and the assay of opium for the four economic alkaloids.
3. Thebaine is a phenanthrene alkaloid of opium -- having the same fundamental structure as morphine and codeine -- and is under international narcotics control. Papaverine, however, -- like narcotine -- does not have the phenanthrene structure and is not under international control; but it is sometimes nationally controlled.
4. The percentages of thebaine and papaverine vary extremely in different types of opium. It is therefore expected that the determinations will be useful in solving the enforcement problem of the origin of illicit opium, although the study of known samples is still quite incomplete.
5. The four economic alkaloids are also the four principal alkaloids in smoking opium, since most of the narcotine is discarded in the preparation of opium for smoking purposes. The best chance of determining the origin of the original opium by chemical means, when it has been made into smoking opium, therefore seems to rest with determinations of the relative amounts, or ratios, of these four alkaloids, thebaine and papaverine as well as morphine and codeine.
6. The new methods will therefore be useful in the commercial evaluation of different types of opium; in analytical "control" of the factory extraction of opium alkaloids; possibly in the control and accounting of narcotic drugs by national and international authorities; and in the problem of determining the origin of illicit opium by scientific means.

Discussion

7. Of the five major alkaloids of opium, narcotine and papaverine are, in general, the easiest to extract with organic solvents, morphine and codeine the most difficult,

and thebaine in between. Morphine at one extreme can be separated from the other major alkaloids by means of its solubility in alkali. So can narcotine at the other extreme, by causing its lactone ring to open. Papaverine, thebaine, and codeine, however, are all non-phenolic, non-acidic alkaloids of generally similar properties in regard to extractions, differing in degree rather than kind in such properties.

8. Since thebaine is between papaverine and codeine in extractive properties, two distinct and difficult separations are involved in its determination.

9. Papaverine also has extractive properties between two other alkaloids, thebaine on the one side and narcotine on the other. It most resembles narcotine; but the characteristics of narcotine are changed by causing its lactone ring to open, and its separation from papaverine can then be rather easily effected. Therefore the separation of papaverine from thebaine is the more difficult to make complete, and accomplishing it makes it possible and even convenient to determine both thebaine and papaverine on the same sample of opium.

10. The first problem is to get the alkaloids out of the solid opium.

11. Thebaine and the alkaloids more easily extracted (narcotine and papaverine of the major alkaloids) can be taken out of dried, powdered opium by chloroform, using a Soxhlet extractor. In fact, tentative methods along this line were offered in E/CN.7/117/Add.2, 22 September 1948. However, it has not been proved that this extraction is always complete for thebaine.

12. Another method is to extract the alkaloids from the solid opium by means of acidified water. This has the advantage that it is not necessary first to dry and powder the opium. It is also the most general method for getting the alkaloids into solution, so that by varying the later procedure, any of the other alkaloids of opium might be determined on the same extract.

13. An extraction of all the alkaloids is best accomplished with rather strong acetic acid. The mineral acids (hydrochloric and sulfuric have been generally used) change the "porphyroxine-meconidine" into a red compound; and if such acids are diluted they do not readily dissolve the narcotine crystals; and they do not disintegrate the opium itself nearly as readily as acetic acid does. As no narcotine determination is included in the present mehtod, a strength of 5 per cent acetic acid is sufficient for the aqueous extraction.

14. The next problem is, what specific method, in particular what solvent, is to be used for separating the thebaine from the codeine, or from the papaverine?

15. Several combinations of acids and solvents can be used. From unbuffered acetic acid solution carbon tetrachloride will gradually take out narcotine and papaverine but not thebaine; if the solution is appropriately buffered with sodium acetate, thebaine can also be shaken out while the codeine is retained. Chloroform, a more effective solvent, will extract thebaine, as well as narcotine and papaverine, but not codeine, from unbuffered acetic acid. Dilute sulfuric acid will retain thebaine fairly well even against chloroform, while permitting the extraction of narcotine and papaverine.

16. Carbon tetrachloride has much more tendency than chloroform to form emulsions, and if it is to be used for the major separations of the non-phenolic alkaloids, a preliminary solvent extraction of the aqueous extract of opium is necessary; e.g., by continuous extraction with ether from an alkaline medium. Chloroform, on the other hand, can be used for direct extraction of the original opium solution, and the same chloroformic solution can then be used for the major separations.

17. Another principle to be noted is the following: In separating an alkaloid from the solution of opium extractives, simply carrying it along in the original solvent, past various types of aqueous washing and cleaning solutions, is not sufficient for purification; -- the alkaloid should at some time be back-extracted into aqueous solution, in order to free it from non-alkaloidal substances which may be carried along in the organic solvent. After the back-extraction of thebaine from chloroform, the solvent can then be changed to carbon tetrachloride, which will not extract so much extraneous material as chloroform. A back-extraction of papaverine is also necessary, and here also the final extraction is made with carbon tetrachloride.

18. In working out the method, a difficulty was encountered in testing for completion of the thebaine extractions: some other substance is present which gives nearly the same colour reaction with (2 / 1) H_2SO_4, the reagent previously used in testing for the elimination of thebaine in the codeine determination (E/CN.7/202, 18 September 1950). The other substance seems not to be extracted to any extent from opium by lime water, but there is quite a quantity of it in the acetic-acid extract. Thebaine gives almost as strong a colour with syrupy H_3PO_4 as with (2 / 1) H_2SO_4, and a fairly strong orange even with concentrated HCl;

the other substance yields a very weak reaction with H_3PO_4, and almost nothing with HCl. The presence of thebaine can therefore be recognized by these latter reagents.

19. Finally, the question of how much opium to use, must be answered. Having regard to the usual scale of laboratory procedures, the more opium that can be used conveniently, the better. At the same time the amounts of the alkaloids involved should be small enough not to require excessive quantities of solvents. The thebaine in 3 grammes opium is generally enough for a convenient titration with n/50 acid, and this amount of opium was selected for use in the method.

Principles of the Thebaine separation

20. The solid opium is extracted with dilute acetic acid, and the filtered solution is slightly buffered with sodium acetate, which tends to convert alkaloidal meconates into acetates. The solution is extracted with chloroform. The chloroform soon takes out all the narcotine, papaverine, and thebaine, along with some amounts of the minor alkaloids, including most of the cryptopine and protopine. A considerable part or even most of the codeine is also extracted.

21. The chloroform extracts are shaken first with three solutions of unbuffered acetic acid. These acid solutions take out the codeine, any traces of morphine that may have been extracted from the original opium solution, part of the cryptopine and other minor alkaloids, and a considerable amount of other impurities, including a considerable part of the one which yields a thebaine-like reaction with sulfuric acid. The completion of thebaine extraction is tested for with concentrated HCl.

22. The chloroform extracts are then washed with two alkali solutions. These remove a considerable amount of colored impurities, and the acidic alkaloid narceine. Any "alkaloid 3" (a minor phenolic alkaloid) that may have been carried this far, is likewise removed, but other minor phenolic alkaloids that are or may be present, are removed only partially, if at all, at this stage. The alkali solutions also have the necessary function of taking out acetate from the chloroform so that in the next stage the remaining alkaloids may be separated by means of chloroform and dilute sulfuric acid.

23. The chloroform-extracts are then shaken with three solutions of dilute sulfuric acid, and this is the important stage at which thebaine is separated from the narcotine and papaverine. Fresh chloroform is used as long as necessary to

shake out all the narcotine and papaverine from the acid solutions. Any cryptopine, protopine, and "porphyroxine-meconidine" still in the chloroform at the beginning of this stage are left in the dilute acid solutions with the thebaine. The chloroform extracts are then shaken with dilute bicarbonate solution to eliminate any acidity. and reserved for the papaverine determination (paragraph 29).

24. The three solutions containing the thebaine in dilute sulfuric acid are washed with carbon tetrachloride, preparatory to a change of solvent, and combined. They are then made strongly alkaline with sodium hydroxide, and shaken out with carbon tetrachloride past an alkali wash. This treatment removes the greater part of the minor phenolic alkaloids still present, as well as other impurities that are carried past alkali by chloroform but not by carbon tetrachloride.

25. The CCl_4 extracts are then shaken with two solutions of dilute acetic acid buffered with sodium acetate. These aqueous solutions take out any codeine that may have got so far, most of the remaining cryptopine and part of the protopine, and most of the remaining impurity with the thebaine-like colour-reaction with sulfuric acid. The extracts are then shaken with dilute ammonia, washed with water, and run into a weighed dish, and the solvent evaporated off.

26. Generally, the separated thebaine crystallizes very readily beginning to do so on the sides of the dish even before the solvent has evaporated completely. In a few cases, which seem to be most often samples of Turkish or Yugoslav origin, crystallization-hindering impurities are still present. The samples of highest thebaine percentages (from Indochina and Iran) have yielded completely crystalline thebaine residues.

27. In all cases the residues have been titrated. The titration result is always somewhat lower than the weight result, more so when the thebaine refuses to crystallize than when it crystallizes completely, as it usually does.

28. If desired, the thebaine can be destroyed by boiling with strong hydrochloric acid (forming the phenolic thebenin) and the unchanged non-phenolic alkaloids extracted from alkaline solution. Only a little residue with a small titration, equivalent to less than a tenth of a per cent of the opium weight, has been found in the thebaine, in the tests that have been made in this way since the method was put in its present form. No correction has been applied to these thebaine values because it is thought that the losses of thebaine amount to at least as much. The other alkaloid thus found in the thebaine seems to be mainly protopine rather than

cryptopine. The latter is slightly closer to codeine in properties, than is protopine.

Principles of the Papaverine separation

29. The extraction from opium and the separation of papaverine and narcotine from the other opium alkaloids, and in particular from thebaine, have already been discussed (paragraphs 20 to 25). Chloroform is recovered from the solution containing the narcotine and papaverine by distillation to a comparatively small volume, and then the solution is poured into a dish and the remaining chloroform evaporated off.

30. The residue of alkaloids then heated in alcohol with potassium hydroxide. This opens the lactone-ring of narcotine and makes it alkali-soluble in water, and insoluble in carbon tetrachloride. The alcoholic solution is poured into water and the papaverine extracted with carbon tetrachloride.

31. It will be noted, however, that up to this point the papaverine has been extracted by the organic solvent in every case. Therefore, it is now back-extracted from the carbon tetrachloride by means of dilute sulfuric acid, and the carbon tetrachloride is reserved for recovery by distillation.

32. The acid solutions are then combined, made alkaline, and extracted with fresh carbon tetrachloride, past a small solution of unbuffered dilute acetic acid. This acid solution is precautionary and intended to hold back any thebaine that might have got into the narcotine-papaverine residue. The carbon tetrachloride extracts are then shaken with dilute ammonia and washed with water and filtered into a weighed dish. Fresh carbon tetrachloride is used as necessary to extract any papaverine held by the unbuffered acetic acid solution.

33. Thus far, the papaverine has been determined by weighing only, not by titration. However, the residue has been completely crystalline in all cases when any considerable amount has been present. The residues have been somewhat yellowish, possibly due to a little papaveraldine (Xanthaline). In a very few cases (two for Indochina and one for Afghanistan) the residue was extremely small and refused to crystallize, and qualitative test indicated that little if any actual papaverine was present. In other cases of low results crystalline papaverine was obtained.

Charts Summarizing the Method

34. Charts 1, 2, and 3 summarize the principles of the thebaine and papaverine separations.

CHART 1

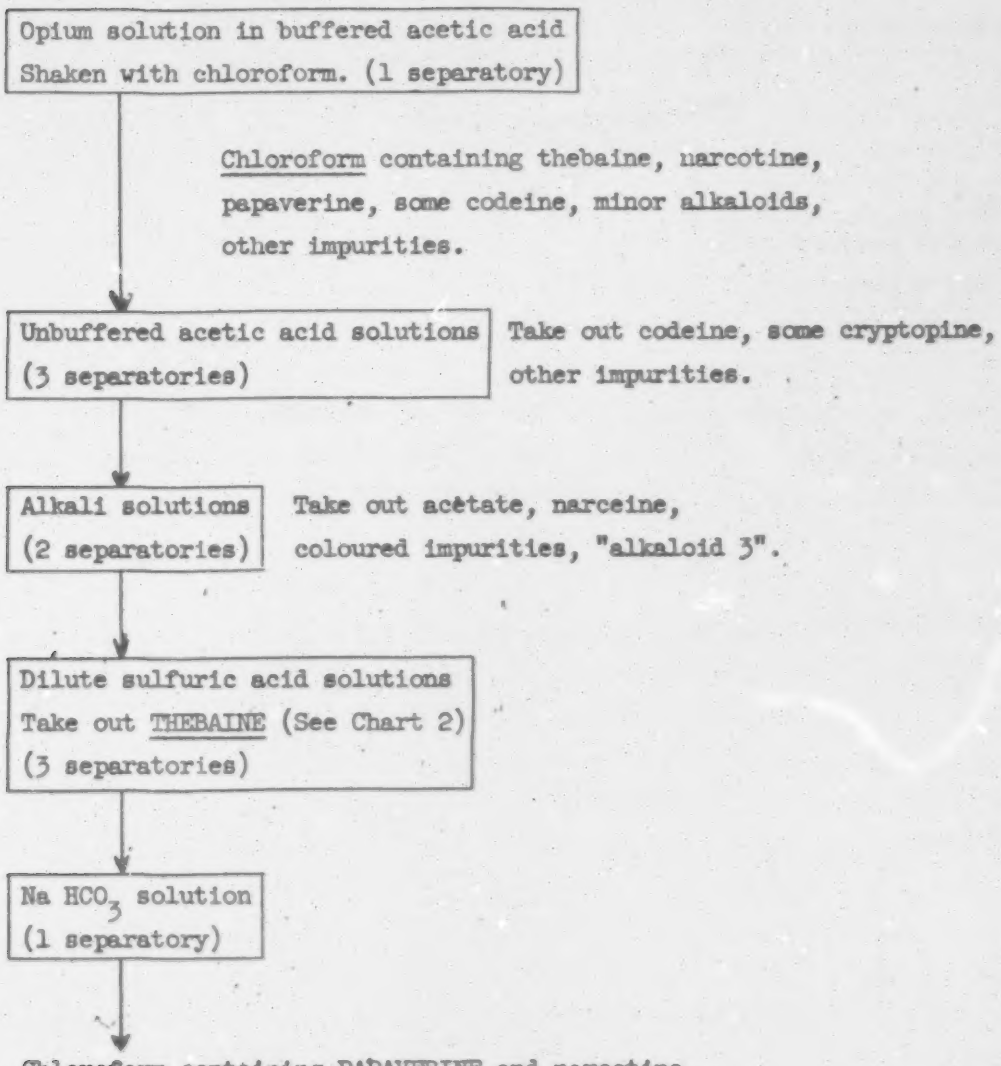

Opium solution in buffered acetic acid
Shaken with chloroform. (1 separatory)
Chloroform containing thebaine, narcotine, papaverine, some codeine, minor alkaloids, other impurities.
Unbuffered acetic acid solutions (3 separatories)
Take out codeine, some cryptopine, other impurities.
Alkali solutions (2 separatories)
Take out acetate, narceine, coloured impurities, "alkaloid 3".
Dilute sulfuric acid solutions
Take out THEBAINE (See Chart 2)
(3 separatories)
Na HCO3 solution
(1 separatory)
Chloroform containing PAPAVERINE and narcotine.
(See Chart 3)

CHART 2

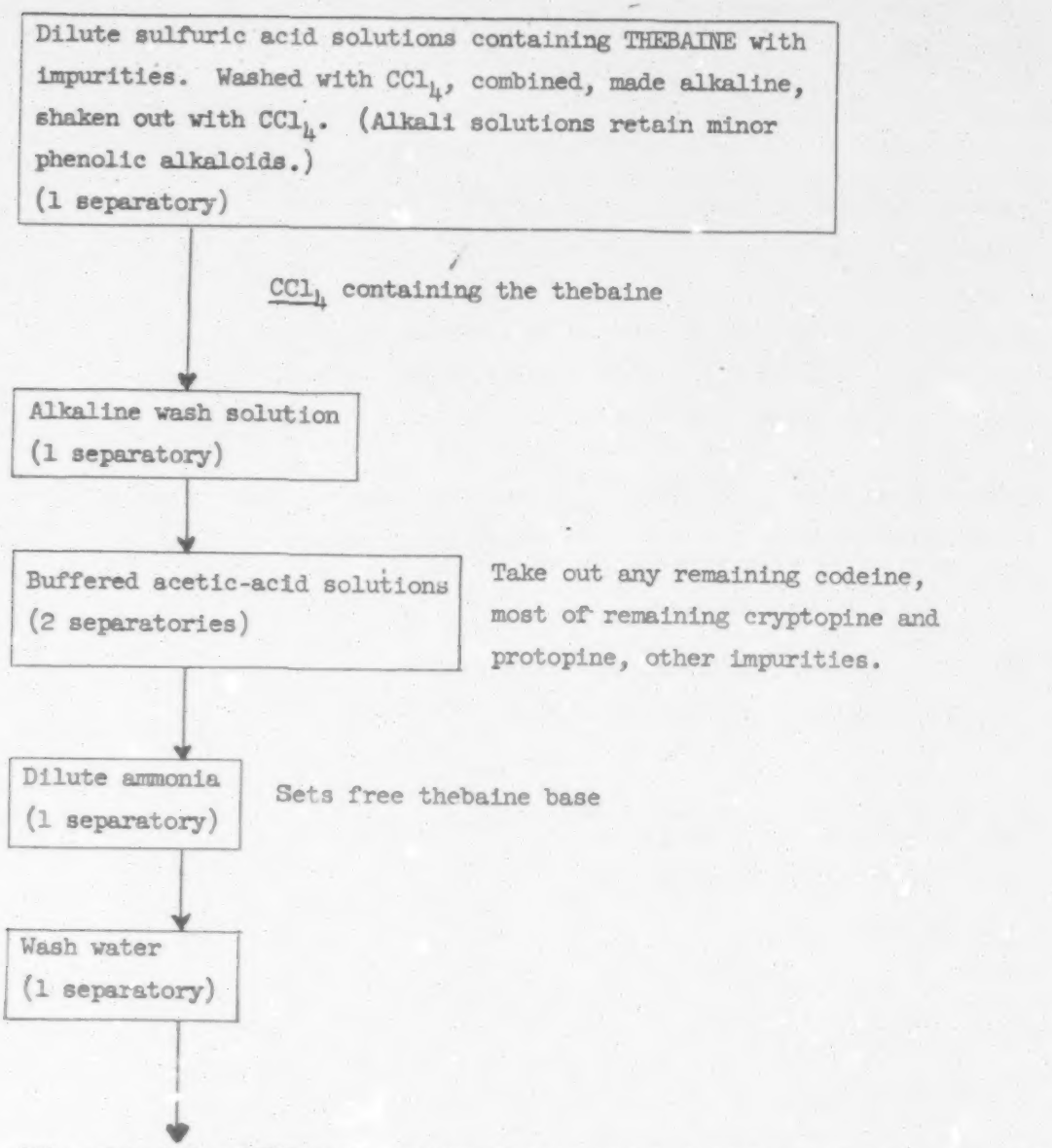
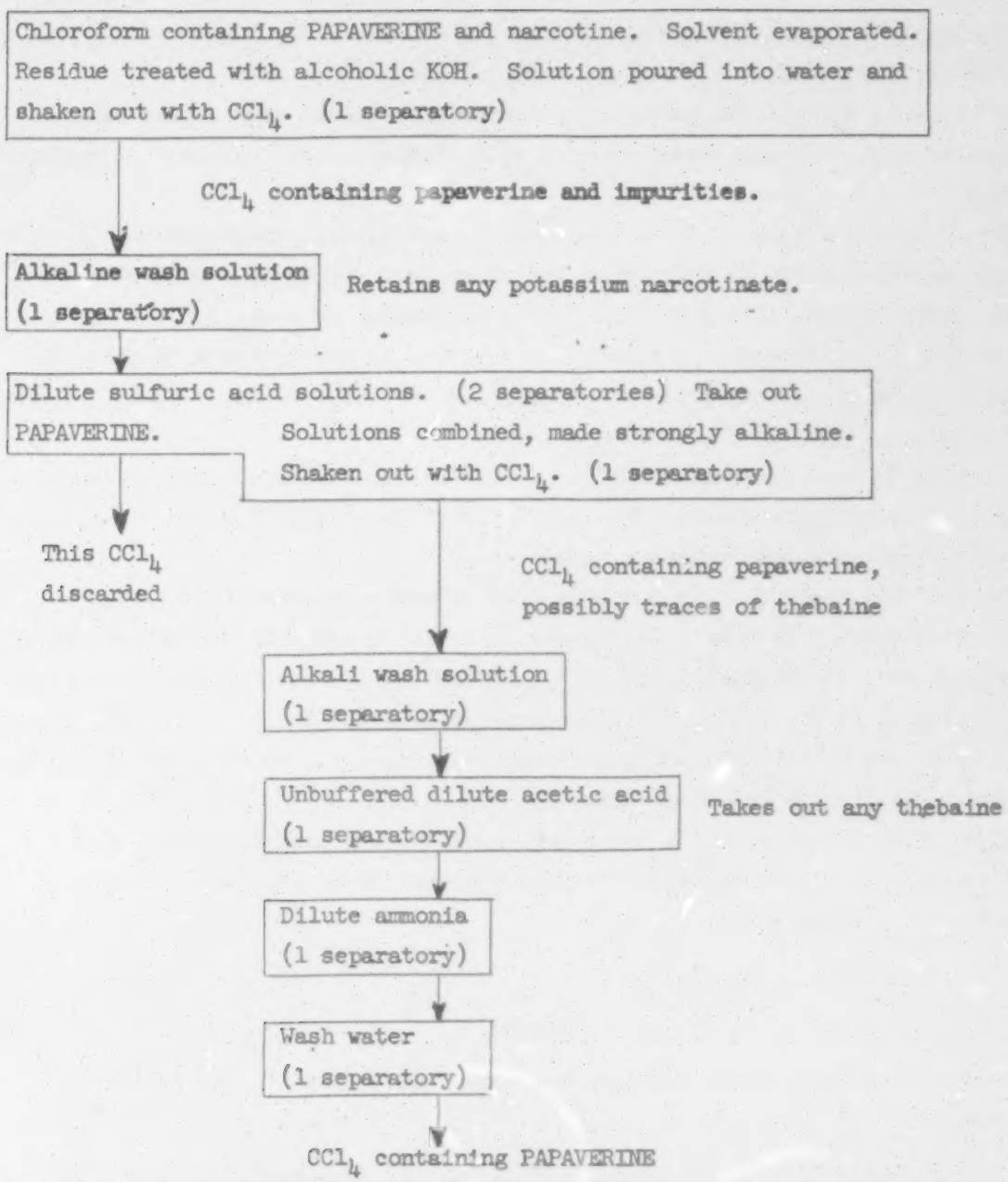

Dilute sulfuric acid solutions containing THEBAINE with impurities. Washed with CCl_4, combined, made alkaline, shaken out with CCl_4. (Alkali solutions retain minor phenolic alkaloids.)
(1 separatory)
CCl_4 containing the thebaine
Alkaline wash solution
(1 separatory)
Buffered acetic-acid solutions
(2 separatories)
Take out any remaining codeine, most of remaining cryptopine and protopine, other impurities.
Dilute ammonia
(1 separatory)
Sets free thebaine base
Wash water
(1 separatory)
CCl_4 containing THEBAINE. Filtered into weighed dish; solvent evaporated on steam bath.

CHART 3


Chloroform containing PAPAVERINE and narcotine. Solvent evaporated. Residue treated with alcoholic KOH. Solution poured into water and shaken out with CCl_4. (1 separatory)
CCl_4 containing papaverine and impurities.
Alkaline wash solution (1 separatory)
Retains any potassium narcotinate.
Dilute sulfuric acid solutions. (2 separatories) Take out PAPAVERINE.
Solutions combined, made strongly alkaline. Shaken out with CCl_4. (1 separatory)
This CCl_4 discarded
CCl_4 containing papaverine, possibly traces of thebaine
Alkali wash solution (1 separatory)
Unbuffered dilute acetic acid (1 separatory)
Takes out any thebaine
Dilute ammonia (1 separatory)
Wash water (1 separatory)
CCl_4 containing PAPAVERINE

Filtered into weighed dish; solvent evaporated on steam bath.

Reagents used for Colour Reactions in the Procedure

35. Concentrated HCl (orange with thebaine).

36. Syrupy H_3PO_4 (85%). (red-orange with thebaine)

37. (2 plus 1) H_2SO_4. Two parts concentrated H_2SO_4 mixed with 1 part water. (orange-red with thebaine, same reaction with another -- an "unknown" -- substance in opium).

38. Buckingham's reagent. 10 to 15 gm ammonium molybdate dissolved in 100 cc of pure concentrated H_2SO_4 by heating on the steam bath. Keep in a well-stoppered bottle. (violet with protopine, green with cryptopine or codeine).

39. Molybdate-SO_3 reagent. A mixture of a few cc of Buckingham's reagent with an equal volume of furning H_2SO_4 containing 15% free SO_3. A new reagent. (purple with thebaine). Make up in small quantities.

40. Furning Frohde's reagent. Mix 0.3 to 0.4 cc Buckingham's reagent with 4 or 5 cc furning H_2SO_4 (15 to 25% free SO_3). (intense violet with papaverine). Use fairly fresh and test on known papaverine.

41. Persulfate in H_2SO_4. About 0.2 to 0.25 gm ammonium persulfate per 10 cc concentrated H_2SO_4. Must be fairly fresh, but the ingredients do not have to be weighed and measured accurately for ordinary tests. (orange-red with narcotine)

42. (3 plus 1) Marquis reagent. 4 drops formaldehyde solution (37 - 40%) mixed with 3 cc concentrated H_2SO_4 and 1 cc water. (purple with codeine but virtually negative to cryptopine and protopine). Use fairly fresh.

43. H_2SO_4 with ferric salt. 3 drops 10% $Fe_2(SO_4)_3$ solution with about 5 cc concentrated H_2SO_4. (strong violet with cryptopine or protopine, virtually negative with codeine).

Caution

44. Carbon tetrachloride is required for some of the steps in the following procedure.

NOTE: IN CONSIDERATION OF THE DANGERS OF CARBON TETRACHLORIDE POISONING, BE SURE THE VENTILATION IS ADEQUATE AND AVOID, SO FAR AS POSSIBLE, BREATHING THE FUMES OF THIS SOLVENT.

Detailed Procedure for Thebaine and Papaverine in Opium

45. The following procedure requires about one day's working time to determine both thebaine and papaverine. It is not short, but neither is it tedious, as several series of different steps are required, each step having its own meaning. The description of the procedure is necessarily long when all the details are given.

46. Weigh 4.5 gm of the opium into a mortar having a pouring lip. Work up with 44 cc of 5% (by volume) acetic acid. Rub the opium marc and stir until solution is as complete as possible. Filter through a 12.5 cm folded filter paper. Take 30 cc of the filtrate (equivalent to 3 gm opium).

Isolation of Thebaine

47. Ten separatory funnels are temporarily necessary. These contain the following solutions:

#1 - The opium solution, with the addition of 1 gm sodium acetate
#2 - 8 cc of 5% acetic acid
#3 - 6 cc of 5% acetic acid
#4 - 5 cc of 5% acetic acid
#5 - 20 cc of 10% NaOH solution
#6 - 20 cc of 2 1/2% NaOH solution
#7 - 14 cc of 1% (by volume) H_2SO_4
#8 - 12 cc of 1% H_2SO_4
#9 - 10 cc of 1% H_2SO_4
#10 - 20 cc water containing a little $NaHCO_3$.

48. Begin the shakeout with 30 cc $CHCl_3$, and continue with 25 cc portions of $CHCl_3$. Each portion is shaken with the opium solution in separatory #1 and then with the other solutions in turn. (An emulsion forms with the opium solution but it will break after standing for some minutes.) From separatory #10 filter the $CHCl_3$ through a pledget of cotton into a flask for recovery of $CHCl_3$ and isolation of the papaverine therein.

49. Make four extractions of the original opium solution and move them along until the fourth extract has been tested, before starting another portion of $CHCl_3$ in separatory #1. Test the fourth extract from separatory #2 for the absence or

continued extraction of thebaine. Add a drop of ammonia to a little of the extract in a small porcelain crucible, evaporate on the steam bath, cool, and apply 3 or 4 drops of concentrated HCl. Thebaine yields an orange colour. A slightly delayed, diffused yellowish colour rather than a small spot which is distinctly orange before being dispersed in the solution, is indicative of the substance yielding a thebaine-like H_2SO_4- reaction, rather than a trace of thebaine.

50. If no thebaine reaction is noted in this test, the contents of separatories #1 and #2 can both be discarded. If only a trace of thebaine is indicated, discard the original opium solution (separatory #1), but start a new portion of $CHCl_3$ with the solution in separatory #2, before discarding it. If thebaine is still coming out in more than a trace, continue the extraction of the original opium solution. If in doubt as to the nature of the test, repeat it on the same extract after shaking with the solution in separatory #3. If it is really due to thebaine, it will be as strong or stronger (from picking up some thebaine left from the preceding extracts); if the colour is only due to the other substance, it will be weaker on the test from separatory #3 (as some of this substance will be left in the aqueous layer). The thebaine should be all out of the original opium solution in four to seven extractions.

51. As soon as the original opium solution has been discarded, begin tests from separatory #5 (the first alkali solution). The acetic acid solutions do not hold any thebaine very long, usually only one extraction after the original opium solution is discarded. When only a trace of thebaine is found by HCl test, the $CHCl_3$ portion is passed on to the dilute H_2SO_4 solution in separatory #7, and the aqueous solutions in the preceding separatories are then discarded. If no thebaine is found in the extract tested from separatory #5, this $CHCl_3$ portion should not be passed on to separatory #7, but simply run into the $CHCl_3$ saved for redistillation, and the aqueous solutions in the separatories preceding then discarded.

52. The chloroform solutions already mentioned, processed to the end of the line of separatories, take with them most of the narcotine and papaverine, while leaving the thebaine in the H_2SO_4 solutions (separatories #7, #8, and #9). It now remains to shake out the last of the narcotine and papaverine from the H_2SO_4 solutions. (This may require as many as 6 or 8 additional extractions.) Start fresh portions of 25 - 20 cc $CHCl_3$ in separatory #7; make tests on the $CHCl_3$ from

separatory #9. Evaporate each test portion with a drop of ammonia, cool the crucibles, and test for narcotine with Persulfate in H_2SO_4 (orange-red colour indicates narcotine) and for papaverine with Fuming Frohde's reagent (papaverine yields an intense violet). Generally the papaverine reaction persists through more shakeouts than that of narcotine because it is more sensitive and the papaverine is harder to extract completely; but since some opiums contain virtually no papaverine, both tests should be tried at first.

53. Continue the extractions until the narcotine and papaverine reactions are absent or negligible. Then discard the $NaHCO_3$ solution. (The papaverine reaction is extremely sensitive, so only a small portion of $CHCl_3$ should be taken for this test, and any doubtful reaction should be disregarded. However if there is the least doubt of the strength of the fuming acid, try out the reagent on a tiny speck of known papaverine.) Run the $CHCl_3$ extracts into the same flask mentioned in paragraph 48.

54. Toward the end of the narcotine-papaverine extraction it may be advisable to test for the non-extraction of thebaine. At this stage, the test can be made with (2 plus 1) H_2SO_4. A new sensitive reagent has been discovered which can also be used. The Molybdate-SO_3 reagent yields a purple colour with thebaine. It cannot be used to test for thebaine in the earlier stages of this procedure, as the unknown substance which yields a thebaine-like reaction with H_2SO_4 gives a strong dark colour, practically black. The Molybdate-SO_3 reagent does not contain enough fuming acid or free SO_3 to yield the Fuming Frohde's reaction with papaverine; papaverine yields a green colour but the reaction is much weaker than the intense violet with Fuming Frohde's. In case leakage of thebaine is noted, the last two H_2SO_4 solutions (in separatories #8 and #9) can each be poured back into the preceding separatory, and a fresh acid solution, 6 to 8 cc of 1% H_2SO_4, put in separatory #9.

55. When the narcotine and papaverine have been removed from the three H_2SO_4 solutions, wash them with two successive portions of CCl_4 to remove residual chloroform. Discard the CCl_4.

56. A new set-up of separatories is now made. The separatories #7, #8, and #9 now become #1, #2, and #3. Pour the acid solution of #2 into #1, and the acid solution of #3 into #2 and then into #1.

Separatory #1 now contains the combined acid solutions, and the following solutions are put into separatories #2 to #6:

#2 - 20 cc of 5% NaOH

#3 - 7 1/2 cc of 5% acetic acid, with 3 gm sodium acetate

#4 - 5 cc of 5% acetic acid, with 2 gm sodium acetate

#5 - 15 cc water plus 1 cc concentrated NH_4OH

#6 - 15 cc water.

57. Now make the combined acid solutions in separatory #1 alkaline with concentrated (1 plus 1) NaOH, and add 2 cc of the alkali solution in excess. Shake out with 30 - 25 cc portions of CCl_4, shaking each portion with the other solutions in turn; however, leave the first portion, or if necessary the first two or three portions, in separatory #6 temporarily, to be run into an evaporating dish a little later.

58. Test the fourth shake-out from separatory #1 for continued extraction of thebaine. Evaporate a little of the CCl_4 in a small porcelain crucible and test the residue with syrupy H_3PO_4. (In case of a doubtfully positive reaction the test can be checked by another test with concentrated HCl; but close the HCl bottle and remove all possibility of HCl fumes in the neighborhood before the CCl_4 extract of the thebaine is run into the final evaporation dish.) As soon as the thebaine is out of the solution in separatory #1 (generally in four extractions), discard it and also the alkali wash-solution in separatory #2.

59. It now remains to extract the last of the thebaine still in the buffered acetic-acid solutions. (This will require only a few more shake-outs.) Filter the CCl_4 from separatory #6 through a pledget of cotton in the stem of an ordinary funnel, into a weighed glass evaporating dish. A dish of 200 cc capacity is most convenient. Continue the extraction with portions of 20 - 15 cc CCl_4.

60. Test the CCl_4 extracts from separatory #5; evaporate a small portion and test with H_3PO_4. When the thebaine is out, discard the solutions in the separatories. Evaporate the collected solvent on the steam bath and heat until the residue is crystalline. Cool in the desiccator and weigh.

61. Dissolve the residue in a little methanol, add two or three drops methyl red indicator, and run in n/50 acid until the solution turns red. Add 2 or 3 cc excess and make sure that all the thebaine is in solution. Dilute with some recently boiled water. Back-titrate with n/50 NaOH, and then add n/50 acid again just to a faint acid colour. (If the N/50 alkali has not been made up fresh, and

is used but seldom, check its strength against the acid before computing the titration.) The cc of acid consumed, multiplied by 0.00629, gives the amount of thebaine. Multiply by 100 and divide by 3 for percentage.

62. The thebaine residue contains just a little protopine, but by this process it should be less than 0.1 per cent of the opium weight, and is probably not even enough to quite balance the slight losses of thebaine.

Determination of the non-thebaine alkaloids in the titrated thebaine residue

63. The following procedure can be used for thebaine isolated by any method, to determine if it contains any considerable proportion of codeine, papaverine, cryptopine, or protopine. It is not used on all thebaine residues, but just often enough to test the method. With the above method, the isolated thebaine should contain no more than about 0.1% of the opium weight of other titratable alkaloids, mainly protopine.

64. Evaporate the titrated solution to about 5 cc (or to dryness on the steam bath, and take it up in 5 cc water). Pour into a small beaker or Erlenmeyer flask. Rinse in with 5 cc concentrated HCl and 5 cc water. Bring to boiling and keep near boiling temperature for about 5 minutes. This converts the thebaine to the phenolic thebenine.

65. Cool the solution and pour it into a separatory funnel. Rinse in with a little water. Add 1:1 NaOH solution gradually (with cooling if necessary) until strongly alkaline. In separatory #2 put an alkaline wash, in separatory #3 water with a very little ammonium salt and a drop of ammonia, and in separatory #4 water. Shake out with CCl_4, shaking separatory #1 very gently, as a bad emulsion is easily formed, and allowing plenty of time for the emulsion to break. Shake the extracts with the solutions in the other separatories and filter into a weighed dish.

66. Completion of the extraction (in the case of thebaine isolated by the method of this paper) can be tested for with H_2SO_4 containing a little ferric salt (intense violet colour with cryptopine or protopine). (For tests useful for codeine and papaverine see paragraph 68 below.)

67. Evaporate off the CCl_4 on the steam bath, dry, cool, and weigh the residue.

Titrate with n/50 or n/100 acid. (A full titration will not be obtained in the ordinary way if the recovered alkaloid is papaverine, as it is a very weak base.)

68. After determining the amount of non-thebaine alkaloids, put a portion of the titrated solution into a separatory funnel and shake out the indicator from slightly acid solution with CCl_4. Then make the solution alkaline or ammoniacal and shake with CCl_4. Evaporate portions for tests. A test for codeine may be made with (3 plus 1) Marquis' reagent; for cryptopine-protopine with H_2SO_4 containing a little ferric salt. Buckingham's reagent yields violet with protopine, green with cryptopine (green also with codeine, but this is not so very sensitive). Papaverine may be tested for with fuming Frohde's reagent in the absence of protopine and cryptopine (which will yield violet with Fuming H_2SO_4 alone). It could also be detected by micro-crystal test. A test with syrupy H_3PO_4 can be used to show that thebaine is no longer present.

Determination of Papaverine in the Chloroform from the Thebaine Determination

69. Connect the flask containing the $CHCl_3$ with the narcotine and papaverine in it (paragraphs 48, 52-53) to a distilling condenser and distill off the $CHCl_3$ for reuse, down to about 50 cc (or less) remaining. Pour the chloroform solution into an ordinary evaporating dish, rinse in with a little $CHCl_3$, and evaporate to dryness.

70. Put 4 pellets of KOH (about 0.4 gram) in a small Erlenmeyer flask with about 11 cc alcohol, cover with a small watch-glass, and warm on the steam bath until the KOH is dissolved. Then pour the alcoholic KOH on the papaverine-narcotine residue, stir it into solution with a glass rod, cover the dish with a watch-glass, and heat on the steam bath for a few minutes. Be sure that all the residue gets into solution and is heated.

71. Partially cool the alcoholic solution, and pour it into 10 cc water in separatory No. 1. Rinse in with 15 cc more water in portions. Rinse the dish also with 30 cc CCl_4, pour this also into the separatory and shake.

72. The following separatories and aqueous solutions are used:

#1 - the alkaline solution (paragraph 71) containing papaverine and potassium narcotinate

#2 - 25 cc wash water

#3 - 15 cc of 2% (by volume) H_2SO_4

#4 - 10 cc of 2% H_2SO_4

73. Shake the CCl_4 extract with each of the solutions in the separatories in turn, then reserve the solvent for redistillation. Shake thoroughly with the H_2SO_4 solutions and allow to settle well, as the papaverine is back-extracted into aqueous solution at this point. Continue the shakout with 25 - 20 cc CCl_4 at a time. Evaporate a small portion of the first CCl_4 from separatory #4 with a drop of ammonia and test the residue with Fuming Frohde's reagent for absence of papaverine. If necessary, add a third acid solution in a fifth separatory. Also test one or two subsequent portions of the CCl_4, to be sure that all the papaverine remains in the acid solutions.

74. Test the 4th CCl_4 extract from separatory #1 for papaverine. (5 extractions at most should be enough.) The test is made with Fuming Frohde's reagent (paragraphs 40, 52, 53). When the papaverine is out of the starting solution, discard it and the wash solution (in separatories #1 and #2).

(Note: narcotine could easily be determined by reconverting the potassium narcotinate. However, it is unlikely that 5% acetic acid, used for the original extraction of the opium, would dissolve all the narcotine. If the original extraction is made with plain water, the determination of the water-soluble narcotine of the opium at this point may be of interest.) Shake the CCl_4 past the acid solutions and reserve it for redistillation.

75. Then move the acid solutions to the first two positions. Pour the 2nd acid solution into the first. Put 4 cc rinse water into separatory #2 and make alkaline with a few drops NaOH solution, then pour into separatory #1. Put 20 cc water in separatory #2. Add three more separatories and solutions for the following set-up:

#1 - the combined acid solutions, containing papaverine

#2 - 20 cc wash water, slightly alkaline

#3 - 5 cc water plus 0.5 cc of 5% acetic acid

#4 - 20 cc water plus 1 cc concentrated NH_4OH

#5 - 20 cc wash water.

(The dilute acetic acid solution is precautionary, to take out any thebaine that may have got so far.)

76. Make the solution in separatory #1 alkaline with 10% or stronger NaOH and shake out with CCl_4, using 25 cc at a time, until the papaverine is out of it, then discard it and the wash solution in separatory #2. Continue with 20 - 10 cc portions of CCl_4 until the papaverine is out of the dilute acetic acid solution.

77. Filter the CCl_4 extracts through a pledget of cotton into a weighed dish, evaporate off the solvent and heat on the steam bath until the whole residue has crystallized. (A little scratching and perhaps a trace of "seed" may help.) Cool in a desiccator and weigh. Multiply by 100 and divide by 3 for the percentage of papaverine in the opium.

Procedure for Papaverine alone

78. When it is not necessary to determine thebaine, the first part of the process, leading to the separation of narcotine and papaverine from thebaine, can be somewhat shortened.

79. Prepare a solution of the opium, using 5% acetic acid, as previously directed (paragraph 46). It is not necessary to buffer this solution with sodium acetate.

80. The separatories and solutions are as follows:

#1 - the opium solution
#2 - 20 cc of 10% NaOH solution
#3 - 20 cc of 2 1/2% NaOH solution
#4 - 10 cc of 2% H_2SO_4 plus 5 cc water
#5 - 6 cc of 2% H_2SO_4 plus 6 cc water
#6 - 5 cc of 2% H_2SO_4 plus 5 cc water
#7 - 20 cc water plus a little $NaHCO_3$

81. Shake out with $CHCl_3$, filtering the $CHCl_3$ from separatory #7 into an Erlenmeyer flask. Thebaine and other substances coming out will mask the papaverine reaction to colour reagents, but 5 shakeouts with $CHCl_3$ portions of 30 - 25 cc should be sufficient to remove all the papaverine from the original opium solution. Then discard the solutions in separatories #1, #2, and #3.

82. Continue the shakeout of the H_2SO_4 solutions with portions of 20 - 15 cc $CHCl_3$, until the papaverine has been extracted. Test small portions from separatory #7 by evaporating the solvent and testing the residue with Fuming Frohde's reagent until the papaverine reaction is negative or insignificant. Then discard the aqueous solutions in the separatories.

83. Continue as previously described, paragraph 69 to 76, except that it may be best to use a slightly stronger acetic acid solution -- 5 cc water plus 1 cc of 5% acetic acid -- in the final shakeout of the papaverine (separatory #3, paragraph 74).

Results

84. A survey of the available samples is under way for their thebaine and papaverine values, but it will be a considerable time before it can be completed. Some representative results are given in Table I.

TABLE I.

Percentages of Thebaine and Papaverine in Opium

COUNTRY	THEBAINE	PAPAVERINE
Yugoslavia U.N. 38 a	1.64	2.42
Yugoslavia 63915 (1938 Export)	0.90	1.60
Yugoslavia, L. of N. V (1935)	0.92	1.99
Greece, U.N. 25 a	1.33	2.67
Turkey, U.N. 15, export type	1.42	1.44
Turkey, 44123 (1944 Export)	1.48	1.23
Turkey, seizure of Standardized opium	1.30	1.38
Iran, U.N. 20, Export	3.24	2.13
Iran, U.N. 47, Fars	4.10	2.12
Iran, sticks, blue green label	2.19	1.93
Afghanistan 63909 (morphine some 15%) (1943 Export)	2.10	0.22
Afghanistan 43533 (morphine some 10%) (1943 Export)	3.54	1.27
India, U.N. 34, Banaras	1.85	0.45
India, U.N. 35, Malwa	1.56	2.09
India, U.N. 36, Excise	1.76	0.66
India, 43385 (1944 Export)	2.30	2.26
Laos, U.N. 27B, Xieng Khouang	2.38	0.80
Vietnam, U.N. 28a, Laokay	4.85	0.10
China, U.N. 37B	1.40	0.84
China, 63423, Manchuria	1.07	1.23
Korea, 63431	2.18	0.65

Concluding Discussion

85. On looking at the method as a whole, it is easily seen that only minor changes would be necessary in order to make it possible to determine narcotine, codeine, and morphine, and perhaps also narceine, on the same sample of opium used for the determination of thebaine and papaverine. These determinations would, however, require a number of changes in the details of the procedure. It is intended to work them out in the future.

86. The nature of the substance yielding a sulfuric-acid reaction similar to that of thebaine has not been established. Further investigation of this substance will be made as soon as time permits.

87. It is of interest that the analysis of a few samples has shown virtually no papaverine (see paragraph 33). This has been reported at least three times before:

(a) L. van Itallie and M. Kerbosch, in examining qualitatively 19 samples of opium from seven different regions of the world for the six principal alkaloids, found all six in all of them except that 4 of the 5 samples from India contained no detectable papaverine. ("Beitrage sur Zusammensetzung des Opiums". Archiv der Pharmazie 248,609-613 (1910).)

(b) K. A. Chassovnikova reported that opium from a certain Chinese variety of Papaver somniferum contained neither papaverine nor narcotine. (Biokhimia 2, 701-704 (1937).)

(c) A. H. Homeyer at Mallinckrodt Chemical Works, St. Louis, U.S.A., in a personal communication a few years ago reported some Afghan opium as containing less than 0.1 per cent papaverine (by his own method of analysis). This is the same type of Afghan opium as found by the Secretariat to contain virtually no papaverine. (The result of the analysis is shown in Table I as 0.22% by weighing, but this residue failed to give any distinct qualitative indications of true papaverine.)

88. The thebaine and papaverine percentages thus far determined do not as yet clearly show any broad geographical classification. Instead, they often reveal subgroups. For example, the two types of opium from Afghanistan, though similar in codeine and porphyroxine content and microscopically, are distinctly different in thebaine and papaverine content as well as morphine percentage. In Turkish opiums, some wide variations have been found from the values for export samples as given in Table I. It should not be surprising that sub-groups in the opium

classification are found, since such producing countries as India and Turkey each recognize different types of opium in the domestic production.

89. At present the use of thebaine and papaverine values for determination of the origin of seized opium seems to lie chiefly in a matching technique: i.e., showing that a seizure corresponds in type -- in general and microscopical appearances and in composition-relations -- with a sample of opium known to originate in a certain country, and that it differs in some essential respects from the opiums known to originate elsewhere. The composition-relations are being worked out at present on the basis of "curves" indicating the morphine, codeine, thebaine, papaverine, and porphyroxine values for each sample.